CONSEQUENCES OF UNILATERAL ULTRAVIOLET RADIATION OF SEA URCHIN EGGS[1]

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The suppression of the elevation of the fertilization membrane on the half of a sea urchin egg which directly receives high doses of ultraviolet light has been described by Reed (1943) and Spikes (1944). The experiments reported herein are an examination of the consequences of unilateral U.V. irradiation of the sea urchin egg in terms of changes in cell morphology with dose, the physical state of the cytoplasm, the effects of time and temperature, and the effects on subsequent cell division. Particular attention is directed toward observations on hyaline layer formation, local gelation, and excentric formation of the mitotic figure.

Materials and Methods

Gametes were obtained from the sea urchin *Strongylocentrotus purpuratus* by injection with 0.5 *M* KCl. The groups of eggs selected were more than 99% fertilizable, were free from visible abnormalities, yielded symmetrical fertilization membranes, and showed little distortion when the lifting of the fertilization membrane began. The pattern of morphological changes at different doses was confirmed with suitable eggs obtained from a single female of the related species *Strongylocentrotus franciscanus,* which has larger eggs with less yolk.

The ultraviolet source was an Electrotherapy Products Corp. low pressure mercury vapor lamp, which produces approximately 95% of its U.V. energy in a 2537 Å band. The intensity was measured with a Hanoviameter.

In some experiments the eggs were centrifuged in a Servall refrigerated angle-head centrifuge, either in sea water or in a sucrose gradient formed by layering sea water over 0.88 *M* sucrose.

Unless otherwise noted, all experiments were carried out in 1 cm. deep, filtered sea water at 17.5 ± 0.1° C. Artificial calcium-free sea water was prepared according to the formula of Moore (1956).

Clarification of terminology

In order to describe concisely and accurately the changes associated with unilateral irradiation of the strongly-absorbing egg certain special terms must be defined. The *directly-irradiated hemisphere* is the surface of the egg which faces

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the U.V. lamp. The *shaded hemisphere* is the surface which does not face the lamp, and, hence, is shaded by the cytoplasm. The *shaded-irradiated axis* is an imaginary line drawn between the *poles* or centers of these two hemispheres. *Unilateral membranes* are fertilization membranes which lift off the egg on the shaded hemisphere only. All drawings and photographs except Figures 1 and 8 have been mounted with the shaded pole facing the top of the page.

Results

When eggs were irradiated with large doses of U.V. and then fertilized, the height of the fertilization membrane and the hyaline layer on the directly-irradiated hemisphere was reduced. Sufficiently large doses unilaterally inhibited the formation of these membranes entirely.

The dose required to produce a definable level of effect varied by as much as a factor of three between the most sensitive and the most resistant groups of eggs. Nevertheless, the ratio of doses necessary to produce two definable effects on the majority of eggs in a population appeared to be constant even in the extreme cases. The data presented represent the most frequently encountered dose relations.

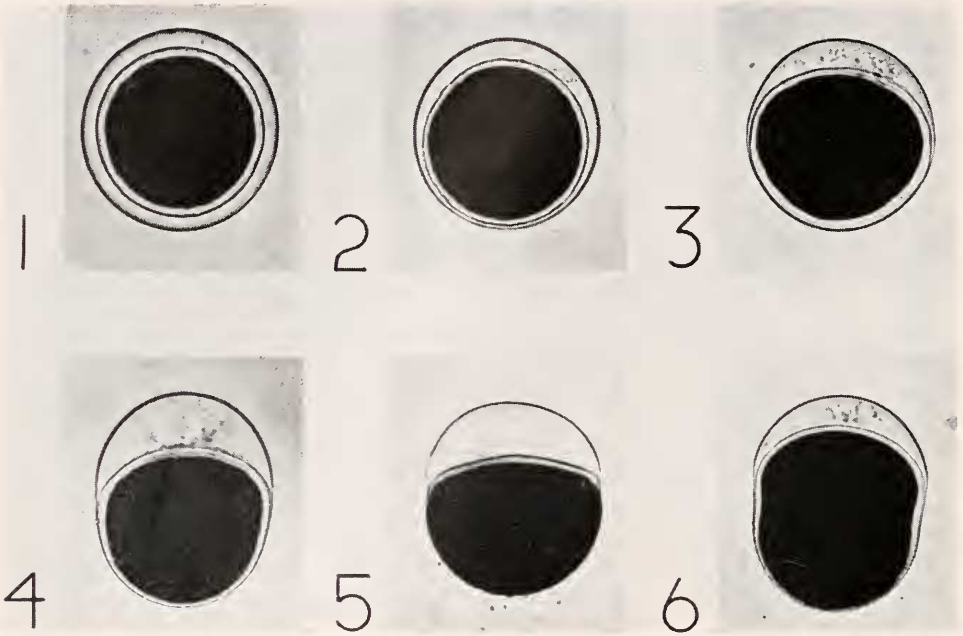
Less than 1600 ergs/mm.^{2} did not interfere with the normal membrane elevation. When the dose was increased the fertilization membranes did not elevate to their normal height over the irradiated pole (Fig. 2). Doses of approximately 2800 ergs/mm.^{2} resulted in the almost complete suppression of the fertilization membrane over a small area, but the hyaline layer differentiated over the entire surface. When the dose was increased to 4800 ergs/mm.^{2} the fertilization membrane covered only one hemisphere, while the hyaline layer appeared normal (Fig. 3). With slightly higher doses a reduction in the thickness of the hyaline layer was sometimes found (Fig. 4). With doses above 7200 ergs/mm.^{2} the hyaline layer could be distinguished only slightly beyond the cell equator (Fig. 5). No further changes in the pattern of membrane elevation were noted at increased doses up to the range of 40,000 to 50,000 ergs/mm.^{2} At this dose level partial cytolysis often occurred immediately on the directly-irradiated hemisphere, and complete cytolysis usually followed after standing or at fertilization.

Identification of the inhibited surface

A simple experimental procedure was devised to demonstrate that the irradiated surface was in fact the one that showed inhibition at fertilization. Stationary eggs were irradiated from above with 7200 ergs/mm.^{2} in a large petri dish on a microscope stage and observed as sperm were carefully added. In four experiments there was no detectable net rotation of any of the eggs in the field of a low power objective. By careful focussing it was established that the fertilization membranes first encircled the lower hemispheres which had been shaded by cytoplasm. As the membranes raised further the eggs rolled over and came to rest on their sides revealing total suppression of membrane elevation on the irradiated hemispheres.

Relationship to time and temperature

Eggs were fertilized at regular intervals from a few seconds after irradiation to as much as twelve hours later without any visible changes in the unilateral



FIGURES 1 to 6

Photomicrographs of sea urchin eggs showing different degrees of suppression of the fertilization reaction when irradiated with increasing doses of U.V. from the direction of the bottom of the page.

FIGURE 1. Control.

FIGURE 2. Reduction of the height of the fertilization membrane.

FIGURE 3. Complete suppression of the elevation of the fertilization membrane on the directly-irradiated hemisphere.

FIGURE 4. Reduction of the height of the hyaline layer.

FIGURE 5. Complete suppression of both fertilization membrane elevation and the hyaline layer differentiation on one hemisphere.

FIGURE 6. Later swelling of the initially flattened shaded hemisphere of an egg similar to Figure 4.

fertilization reaction. In five separate experiments there was no increase or decrease in the inhibited area with time. In general, the irradiated eggs cytolyzed sooner than the controls, but in most experiments both the irradiated and the control eggs became unfertilizable at approximately the same time, even with very high concentrations of sperm.

Attempts were made to re-fertilize eggs which had been fertilized but did not completely differentiate the hyaline layer. The simple addition of viable sperm did not cause re-fertilization at any time up to 28 hours after irradiation. The sperm were observed to accumulate in the egg jelly which adhered to the irradiated hemisphere in each of these experiments.

Irradiating eggs from the same females at 18 and 8° C. with various doses revealed that there were no differences in sensitivity at the two temperatures.

Changes in morphology and physical state of the cytoplasm

The progressive dose-dependent suppression on the elevation of the fertilization membrane and the differentiation of the hyaline layer have already been described. Sometimes at high doses the fertilization membrane was elevated to an abnormal height above the shaded pole and the cytoplasm under it was considerably flattened (Figs. 4 and 5). A large amount of particulate matter, possibly cortical granule materials, was found in the perivitelline space under these conditions. The amount of this material was apparently greater at all doses than in the controls.

After flattening, the cytoplasm under the unilateral membranes sometimes swelled and reduced the thickness of the perivitelline space (Fig. 6). In some cases the thickness was less than the controls. Under these conditions there was a constriction around the cell at the equator where the fertilization membrane met the hyaline layer (Fig. 6).

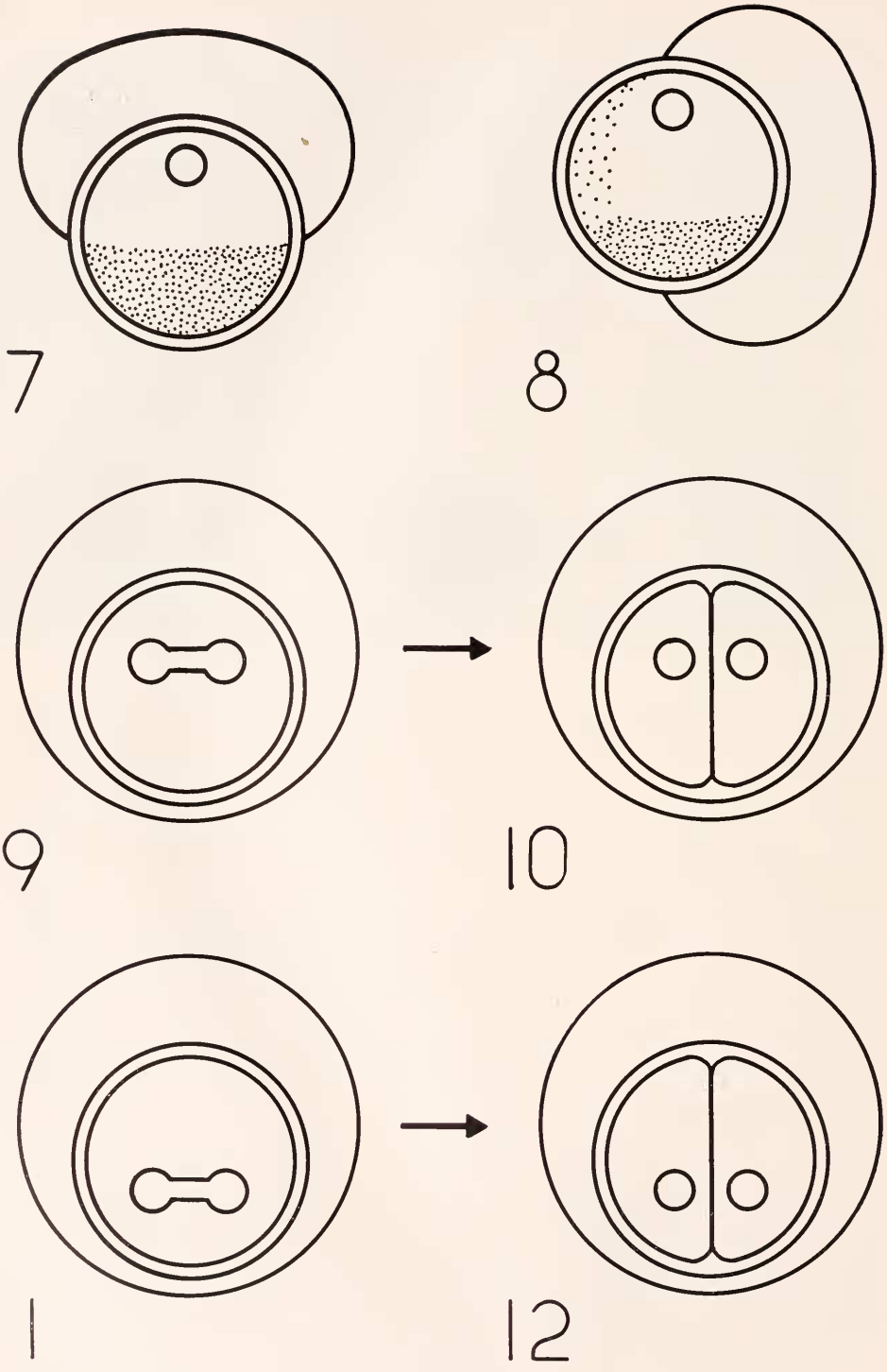
There were no cases of membrane elevation activation by U.V. at any dose in any of the experiments.

Unfertilized irradiated eggs were centrifuged for ten minutes at approximately 12,000 g in a sucrose gradient. In two such experiments 90% of the eggs stratified with the center of the light pole (identified by an oil cap over a clear region of cytoplasm) in the center of the shaded hemisphere (identified by subsequent fertilization) (Fig. 7). Almost all of the remaining 10% had an asymmetry of less than 30° between the light-heavy and the shaded-irradiated axis. A very small fraction of a per cent were 30 to 90° off center, and no cases were found in which the shaded pole appeared to have a greater density than the irradiated one.

When unfertilized eggs were placed in 70% sea water after irradiation they swelled on one pole only, giving the eggs a somewhat pear-shaped appearance. Standing in this hypotonic medium for several hours did not result in any further changes in shape. The treated eggs were fertilized to establish that the shaded pole was the swollen one. Hence, while both unirradiated eggs and the shaded side of an irradiated one swell in 70% sea water, the directly irradiated surface does not.

Irradiated eggs placed in 70% sea water had a dense darkened area near the irradiated pole, a somewhat less dense region at the shaded pole, and a lighter less granular region near the equator. Occasionally this pattern appeared in eggs kept in normal sea water and seemed to be accompanied by a slight enlargement of the shaded hemisphere. With doses of the order of 40,000 ergs/mm.^{2} a large blister of non-granular material formed on the irradiated pole when the eggs were placed in the hypotonic sea water. With slightly higher doses these blisters appeared spontaneously.

Irradiated eggs were centrifuged at approximately 12,000 g in sufficiently dense suspensions that some of the eggs were confined in a random orientation with respect to their light-heavy axes. Some of these cells showed stratification only on the shaded side, which was identified by subsequent fertilization. When the direction of centrifugation was perpendicular to the shaded-irradiated axis there was a narrow region near the irradiated surface with a very high gel strength that resisted stratification when the central cytoplasm and the shaded side stratified (Fig. 8).



FIGURES 7–12

Eggs irradiated after equilibration in calcium-free artificial sea water and fertilized immediately when returned to normal sea water showed the same degree of inhibition as eggs irradiated in normal sea water.

Mitotic abnormalities

Cells irradiated at doses that inhibited the full differentiation of the hyaline layer seldom divided. At lower doses some or all of the eggs would divide several times and sometimes form apparently normal swimming blastulae. Gastrulation was usually abnormal. In some experiments even the first division was abnormal.

A systematic group of abnormalities occurred as a result of the mitotic figure failing to migrate to the center of the egg. The nucleus of the unfertilized egg is excentrically located, and in normal division the mitotic apparatus is *positioned* approximately in the center of the cell. The position of the furrow is determined by the plane formerly occupied by the metaphase plate both in normal cells and these abnormal cells.

When the mitotic figure located in either hemisphere was oriented perpendicular to the shaded-irradiated axis, the furrow formed along that axis and the egg cleaved into two equal-sized blastomeres (Figs. 9 to 12).

When the mitotic figure was oriented parallel to the shaded-irradiated axis in either hemisphere, the furrow formed perpendicular to the axis and the sizes of the resulting blastomeres were quite different (Figs. 13 to 16).

Variable results were observed when the mitotic figure was formed with other orientations with respect to the shaded-irradiated axis (Figs. 17 and 18).

Excentric spindles were also found in eggs which were irradiated during the early part of the first mitotic cycle with comparatively low doses of U.V. The blastomeres in such experiments were always equal in size.

Whenever the mitotic apparatus was excentric the furrow formed first on the surface that was closest to the spindle. At later stages of cytokinesis the furrow on the near side would always be deeper than the furrow on the far side. In some cases the furrow actually passed through the spindle before the first indentation occurred on the far side of the cell.

Discussion

The progressive unilateral inhibition of the fertilization reaction has been described in terms of the U.V. doses required to produce different degrees of inhibi-

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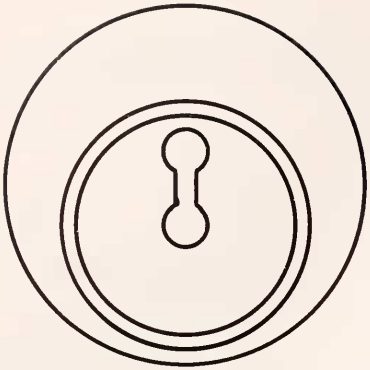
Figures 7 to 18

Schematic drawings of eggs irradiated from the direction of the bottom of the page (except Fig. 8) ; refer to text for explanation.

Figure 7. Egg centrifuged in a sucrose gradient and then fertilized. Stratification direction indicates that the irradiated pole was heavier than the shaded pole.

Figure 8. Egg irradiated from the left side of the page and centrifuged while confined with the shaded-irradiated axis perpendicular to the direction of centrifugation. A narrow region near the surface of the irradiated hemisphere resisted stratification indicating a local increase in gel strength.

Figures 9 to 12. Division patterns of cells with spindles oriented perpendicular to the shaded-irradiated axis.



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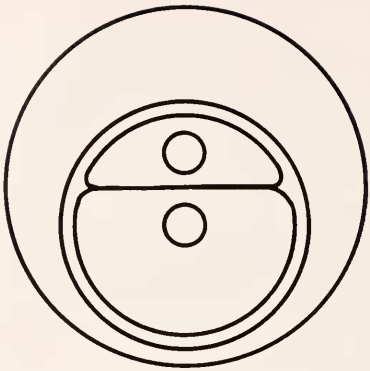

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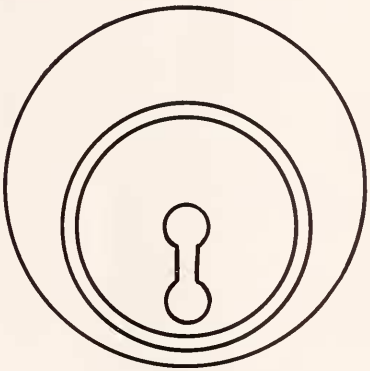

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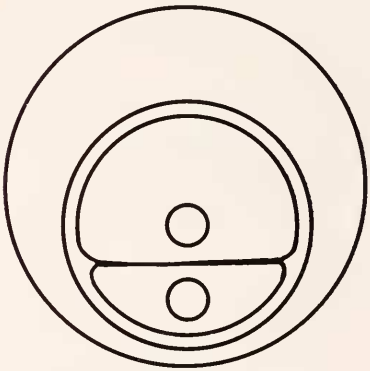

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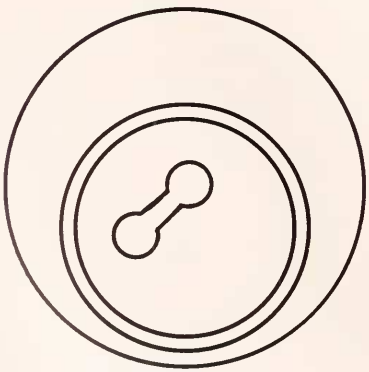

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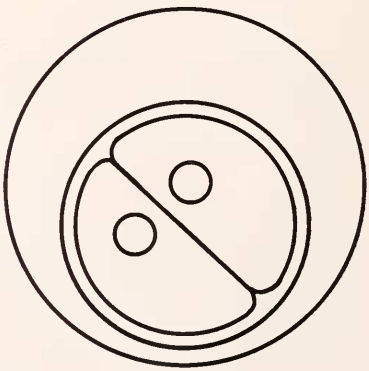

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FIGURES 13–18

tion of both the elevation of the fertilization membrane and the differentiation of the hyaline layer. Hyaline layer differentiation is less sensitive to U.V. than fertilization membrane elevation; however, it may be suppressed completely on the directly-irradiated hemisphere with high doses. The inhibition of the elevation of the fertilization membrane has been described previously by Reed (1943) and Spikes (1944).

By means of local dye experiments Spikes (1944) was able to demonstrate that the directly-irradiated hemisphere is the site of inhibition. His findings have been reconfirmed with the direct observations of undisturbed eggs reported herein. Giese (1947) has shown that the sea urchin egg strongly absorbs or scatters 2537 Å U.V. light. Harvey and Lavin's (1944) U.V. photomicrographs also indicate that a considerable amount of the light is absorbed in sea urchin eggs of another genus. Since the shaded pole is not inhibited even at very high doses, it may be concluded that the transmission of the cytoplasm is too low to allow the necessary energy to reach the sensitive sites on the shaded side of the egg.

The demonstration that there was no spreading of the damaged area with time indicates that the U.V. action is relatively direct, and, in particular, that there is no secondary effect of "diffusible poisons." There was no recovery with time; hence, the damage seems to be irreversible by any metabolic mechanism. Since the degree of injury did not decrease with time, and a diffusible toxic product would be expected to decrease in local concentration, this observation provides additional evidence against the action of such substances.

The sensitivity was the same at 8 and 18° C.

Direct photochemical action has been shown repeatedly to have a Q_{10} of approximately 1. Therefore, insofar as visually equivalent degrees of damage may be used as a measure of the rate of damage, it appears that the injury results from direct photochemical action. The time and temperature relations together offer evidence that the effect is localized and that there is a lack of intermediary toxic products.

The observation that the inhibited surface could not be re-fertilized by the addition of fresh sperm could be interpreted in two ways: either the U.V. damage rendered it unfertilizable or some of the steps of the fertilization reaction occurred on this side when the egg was initially fertilized. If some substances necessary for the initial steps of the reaction had been used up the sperm could not initiate a response later. A pronounced green Becke line appears in the out-of-focus image of the damaged hemisphere of heavily irradiated eggs after fertilization. This change is probably similar to the dark-field changes which have been observed prior to membrane elevation (Runnström, 1928; Rothschild and Swann, 1949) and indicates that some step in the fertilization reaction has taken place.

Two types of evidence for local gelation in the irradiated hemispheres were obtained: first, that swelling in 70% sea water was confined to the shaded pole, and, second, that a narrow band near the irradiated surface resisted stratification with centrifugation when the rest of the cytoplasm stratified. Reed (1948) found

FIGURES 13 to 16. Division patterns of cells with spindles oriented parallel to the shaded-irradiated axis.

FIGURES 17 and 18. An example of one of several division patterns obtained when the spindles have intermediate angular orientations.

that moderate doses of unilateral U.V. did not change the permeability of the egg to a large variety of solutions. Although no measurements were made, he discussed possible differences at higher doses and proposed that some sort of gelation occurred on the basis that vacuoles were formed in the irradiated pole. Spikes (1944) also proposed that gelation occurred, because he found that while normal eggs only swelled in 50% sea water, irradiated ones lysed on the irradiated side.

Spikes' data might also be interpreted as indicating either that the surface of the shaded hemisphere was weakened or that the osmotically inert volume had been increased permitting greater than normal swelling followed by lysis. The observation of the large amounts of granular material released into the perivitelline space at the shaded pole suggests the weakening either of the cell membrane or of some other surface structure. The flattening of the shaded pole at fertilization at high doses seems to fit either hypothesis, although an enhancement of the vigor of the fertilization reaction would yield the same pattern. It would not be unreasonable to suppose that U.V. damage could affect both the surface strength and the osmotically inert volume, perhaps by a common mechanism.

The observation that eggs irradiated in calcium-free sea water showed the same degree of damage as eggs in normal sea water cannot be interpreted directly in terms of the often demonstrated role of calcium in gelation (Heilbrunn, 1952). First, the eggs had to be fertilized in normal sea water since fertilization will not occur in the absence of external calcium ion; hence, new calcium may have been introduced before the damage was measured. Second, since Heilbrunn and his co-workers have shown that U.V. causes solation in low doses and gelation in high doses, it is quite possible that the calcium ion left in the egg after treatment with calcium-free sea water shifts between the less and more heavily damaged portions of the cytoplasm. The second possibility is quite attractive, since it would provide a mechanism for an increase in osmotically inert volume in the less damaged hemisphere and introduces the possibility that the surface on the shaded side might be weakened by small amounts of U.V. penetrating the cytoplasm to cause solation.

Spikes (1944) reported that in *Lytechinus pictus* furrow formation almost always occurs along the shaded-irradiated axis. Clearly this is not the case in the *Strongylocentrotus purpuratus* used in these experiments; cleavage may take place with any orientation. Successful cleavage with the furrow passing through the irradiated portion of the egg indicates either that the furrowing strength exceeds the resistance of the radiation-induced gel or that the gel is solated in the course of cytokinesis.

Cleavage into equal or unequal sized blastomeres is determined by the orientation of the spindle with respect to the shaded-irradiated axis. It occurs because the mitotic figure remains centered around the original location of the nucleus. The nucleus is excentrically located in unfertilized eggs of this species. When the axis of the mitotic figure is perpendicular to the shaded-irradiated axis the blastomeres are equal in size. Where the axes are parallel the blastomeres are unequally sized. In intermediate angular orientations the results are variable. While both parallel and perpendicular orientations can occur when the mitotic figure is located in either the shaded or irradiated hemisphere, mitotic figures near the equator seem to be restricted to intermediate angular orientations. It is clear that the migration of the nucleus to its normal central position is inhibited. An

increase in cytoplasmic viscosity would provide a plausible explanation for this failure of migration.

It is a great pleasure to acknowledge my gratitude to Professor Daniel Mazia for his helpful advice and encouragement during the course of this work. I also wish to thank Professors J. E. Gullberg, L. V. Heilbrunn and C. B. Metz for their valuable comments about the results, and Mr. Fred Burnet for his skillful preparation of the drawings.

Summary

1. The progressive dose-dependent inhibition of the fertilization reaction on the directly-irradiated hemisphere of the unilaterally U.V.-irradiated sea urchin egg has been described in terms of changes in the ability to elevate the fertilization membrane and to differentiate the hyaline layer.

2. Membrane elevation was not activated by 2537 Å U.V. light.

3. No spreading of the extent of injury or recovery was found with time; and no temperature sensitivity differences were found; hence, the injury appeared to be the result of direct photochemical action.

4. The irradiated hemisphere of the fertilized egg maintained its jelly for considerable periods of time.

5. Evidence was obtained showing partial gelation of the irradiated hemisphere and suggesting that the gelled cytoplasm had a higher density than the rest of the egg. Irradiation in calcium-free sea water did not change the degree of damage observed after fertilization in normal sea water.

6. The behavior of the cytoplasm of the shaded hemisphere at fertilization suggested either that the surface structure was damaged or that the osmotically inert volume had been increased.

7. Unilateral irradiation caused excentric spindle formation which resulted in equal sized blastomeres if the spindle axis was perpendicular to the axis of irradiation and unequal sized blastomeres if the axes were parallel.

LITERATURE CITED

Giese, A. C., 1947. Radiations and cell division. *Quart. Rev. Biol.*, **22**: 253–282.

Harvey, E. B., and G. I. Lavin, 1944. The chromatin in the living *Arbacia punctulata* egg and the cytoplasm of the centrifuged egg as photographed by ultraviolet light. *Biol. Bull.*, **86**: 163–168.

Heilbrunn, L. V., 1952. An Outline of General Physiology. Third ed. W. B. Saunders Co., Philadelphia.

Moore, A. R., 1956. *In:* Formulae and Methods IV, Marine Biological Laboratory, Woods Hole, Massachusetts.

Reed, E. A., 1943. Unilateral membrane formation in the sea urchin egg treated with ultraviolet light. *Anat. Rec.*, **87**: 467.

Reed, E. A., 1948. Ultraviolet light and permeability of sea urchin eggs. *J. Cell. Comp. Physiol.*, **31**: 261–280.

Rothschild, Lord, and M. M. Swann, 1949. The fertilization reaction in the sea urchin egg. A propagated response to sperm attachment. *J. Exp. Biol.*, **26**: 164–176.

Runnström, J., 1928. Die Veränderungen der Plasmakolloide bei der Entwicklungserregung des Seeigeleies. *Protoplasma*, **4**: 388–514.

Spikes, J. D., 1944. Membrane formation and cleavage in unilaterally irradiated sea urchin eggs. *J. Exp. Zool.*, **95**: 89–103.